ISSN 1936-8798/$36.00
PUBLISHED BY ELSEVIER INC.
DOI: 10.1016/j.jcin.2010.07.012

CLINICAL RESEARCH

Pilot Study of the Antiplatelet Effect of Increased Clopidogrel Maintenance Dosing and Its Relationship to *CYP2C19* Genotype in Patients With High On-Treatment Reactivity

Colin M. Barker, MD,* Sarah S. Murray, PhD,‡ Paul S. Teirstein, MD,†‡
David E. Kandzari, MD,† Eric J. Topol, MD,†‡ Matthew J. Price, MD†‡

Houston, Texas; and La Jolla, California

Objectives The objective of this study was to evaluate the antiplatelet effect of clopidogrel 150 mg/day in patients with high on-treatment reactivity (OTR) and to further assess this effect according to *CYP2C19* genotype.

Background High OTR is associated with ischemic events in clopidogrel-treated patients after percutaneous coronary intervention. Alternative dosing regimens might enhance platelet inhibition.

Methods Patients with high OTR receiving a standard clopidogrel regimen were identified with the VerifyNow P2Y12 assay and administered clopidogrel 150 mg daily for 7 days, after which OTR was reassessed. Comprehensive *CYP2C19* genotyping was performed with the BeadXpress platform (Illumina, San Diego, California) for the *2, *3, *4, *5, *6, *7, *8, and *17 variants.

Results A total of 41 subjects were enrolled, 20 of whom were carriers of a *CYP2C19* loss-of-function (LoF) allele. High-dose clopidogrel significantly reduced OTR from 285 $\pm$ 47 $P2Y_{12}$ reaction units (PRU) to 220 $\pm$ 91 PRU ($p < 0.001$). There were no significant differences in antiplatelet effect according to *CYP2C19* status, although the reduction in reactivity was minimal in the small number of patients homozygous for LoF alleles (n = 3, 28 $\pm$ 31 PRU, p = NS). Increasing body mass index was independently and negatively associated with the reduction in OTR ($p = 0.009$).

Conclusions In patients with high OTR, clopidogrel 150 mg/day results in a significant reduction in platelet reactivity. Carriage of an LoF *CYP2C19* polymorphism does not seem to have a major influence on dose effect. The observed lack of effect in patients with 2 copies of a *CYP2C19* LoF allele must be confirmed by larger studies. (J Am Coll Cardiol Intv 2010;3:1001–7) © 2010 by the American College of Cardiology Foundation

From the *Division of Cardiology, University of Texas Medical School at Houston and Memorial Hermann Heart and Vascular Institute at the Texas Medical Center, Houston, Texas; †Division of Cardiovascular Diseases, Scripps Clinic, La Jolla, California; and the ‡Scripps Translational Science Institute, La Jolla, California. Dr. Barker is a consultant and has received speaker honoraria from Cordis and Medtronic. Dr. Murray reports that he has no relationships to disclose. Dr. Teirstein is a consultant and has received research grant support from Abbott Vascular, Medtronic, and Boston Scientific. Dr. Kandzari has received research grant support from Abbott Vascular, Cordis, and Medtronic and is a consultant for Eli Lilly, Medtronic, Cordis, and Abbott Vascular. Dr. Topol is a consultant for Quest Diagnostics and Sanofi-Aventis and is supported by National Institutes of Health/National Center for Research Resources-Clinical and Translational Science Awards Grant ULI RR025774. Dr. Price has received research grant support from Bristol-Myers Squibb, Sanofi-Aventis, and Accumetrics; is a consultant for Bristol-Myers Squibb, Sanofi-Aventis, Accumetrics, Boston Scientific, and Eli Lilly; and has received speaker honoraria from Boston Scientific and Eli Lilly.

Manuscript received May 5, 2010; revised manuscript received July 9, 2010, accepted July 25, 2010.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 3, NO. 10, 2010
OCTOBER 2010:1001–7

When added to aspirin, clopidogrel reduces the incidence of thrombotic vascular events among patients with acute coronary syndromes and those undergoing percutaneous coronary interventions (PCI) (1). However, many patients taking dual antiplatelet therapy still experience thrombotic events. The pharmacodynamic effect of clopidogrel varies widely among individuals (2). Patients with high on-treatment reactivity (OTR) are at an increased risk for cardiovascular events after PCI, including stent thrombosis (3–11). Therefore, investigation of the pharmacodynamic effect of alternative dosing regimens in these patients is warranted.

See page 1008

Clopidogrel is a prodrug that undergoes hepatic biotransformation to its active metabolite by several enzymes of the cytochrome P450 family, including *CYP2C19* (12,13). Genetic variants of *CYP2C19*, occurring as either hetero- or homozygous alleles, impede the biotransformation of clopidogrel to its active metabolite and seem to be important determinants of a diminished antiplatelet effect (14–18). The *CYP2C19* genotype might therefore influence the incremental response to high-dose clopidogrel therapy in patients with high OTR to standard dosing.

Abbreviations and Acronyms

ADP = adenosine diphosphate
BMI = body mass index
CAD = coronary artery disease
DNA = deoxyribonucleic acid
LoF = loss-of-function
OTR = on-treatment reactivity
PCI = percutaneous coronary intervention
PPI = proton-pump inhibitor
PRU = P2Y12 reaction units

In this study, we tested the hypothesis that high-dose clopidogrel maintenance therapy would reduce platelet reactivity in patients with high OTR. We further examined the interrelationship between loss-of-function (LoF) variants of the *CYP2C19* gene, platelet suppression, and high-dose therapy in these patients.

Methods

Subjects and study design. This was an observational, longitudinal, open-label, single-center study. Patients with coronary artery disease (CAD) were eligible for enrollment if they: 1) had received maintenance clopidogrel 75 mg/day for ≥7 days or, if taking maintenance therapy for <7 days, received a loading dose of clopidogrel ≥300 mg at the time clopidogrel therapy was initiated; and 2) had high OTR, defined as Verifynow P2Y12 reaction units (PRU) ≥235. Patients were excluded if they had been treated with a glycoprotein IIb/IIIa inhibitor within 1 week; had active bleeding or history of recent bleeding diathesis (within 3 months), a previous hemorrhagic stroke, intracranial neoplasm, platelet count <100,000, hemoglobin <10 g/dl, intolerance to clopidogrel; or were being treated with oral anticoagulation. This study complied with the Declaration of Helsinki and was approved by the Scripps Clinic Institutional Review Board. Informed consent was obtained from all patients.

Study drug administration. Patients were administered clopidogrel 150 mg/day for 7 days, after which platelet reactivity was reassessed. Compliance was assessed with pill counting.

Platelet function measurements. The magnitude of on-treatment platelet reactivity was measured with the VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California). The VerifyNow System is a turbidimetric-based optical detection system that measures platelet-induced aggregation. The assay device contains a lyophilized preparation of human-fibrinogen-coated beads, platelet activators, and buffer. The VerifyNow P2Y12 assay contains 20 μmol adenosine diphosphate (ADP) and 22 nmol prostaglandin E1 to reduce the activation contribution from ADP binding to $P2Y_{12}$ receptors. The VerifyNow instrument measures platelet-induced aggregation as an increase in light transmittance and uses a proprietary algorithm to report values in PRU. With this assay, a higher PRU reflects greater ADP-induced reactivity. Several prospective studies have demonstrated the ability of the VerifyNow P2Y12 assay to identify patients at risk for cardiovascular events after PCI (6,19). "High OTR" was defined as a PRU ≥235. This cut-point has been demonstrated by receiver-operator characteristic curve analysis to predict 6-month ischemic events after PCI with drug-eluting stents and is similar to cutoffs that predict 30-day and 1-year major adverse cardiac events after PCI (3–6,20).

Genetic methodology. DEOXYRIBONUCLEIC ACID (DNA) ISOLATION AND GENOTYPING. DNA was isolated from 200-μl aliquots of frozen blood with the Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia, California) and QiaCube Robotic workstation for automated DNA purification. The *CYP2C19* genotyping for the *2, *3, *4, *5, *6, *7, *8, and *17 single nucleotide polymorphisms was performed with the Illumina *CYP2C19* Panel and Fast Goldengate assay on the BeadXpress Platform (Illumina, San Diego, California). Samples were run in duplicate along with 5 positive control samples that have been previously characterized for *CYP2C19* genotypes with Sanger sequencing. The resulting genotypes had 100% reproducibility (47 of 47 matching calls across 42 patient samples and 5 control samples), 100% concordance with Sanger sequencing results for the 5 positive control samples, and 100% sample call rates for all loci and samples. The *1 allele was assigned when none of the assayed alleles were present.

Table 1. Baseline Clinical Characteristics of Study Population (n = 41)

Age (yrs)	66.6 ± 10.6
Male sex	35 (85.3%)
Diabetes mellitus	16 (39%)
Hypertension	36 (87.8%)
Hypercholesterolemia	36 (87.8%)
Previous cerebrovascular accident	4 (9.8%)
Previous myocardial infarction	13 (31.7%)
Previous coronary artery bypass surgery	15 (36.6%)
Previous PCI	33 (80.5%)
Concomitant proton-pump inhibitor use	10 (24.3%)
Body mass index (kg/m^2)	29.3 ± 5.6
Creatinine clearance (ml/min)	80.5 ± 32.6
Index on-treatment reactivity (PRU)	285 ± 47

PCI = percutaneous coronary intervention; PRU = P2Y12 reaction units.

CLASSIFICATION BASED ON PREDICTED METABOLIC PHENOTYPE. Individual variants of the *CYP2C19* gene were classified a priori according to their predicted metabolic phenotypes (ultra-rapid, extensive, intermediate, or poor enzymatic function) according to published data (21) and with the use of the established common-consensus "star allele" nomenclature (22). The *17/*17 or *1/*17 genotypes were classified as ultra-rapid metabolizers; *1/*1 were classified as extensive metabolizers; the combination of *1 with a LoF allele *2, *3, *4, *5, *6, *7, or *8 as intermediate metabolizers; and any combination of 2 LoF alleles were classified as poor metabolizers. It was determined prospectively to exclude genotypes with the combination of 1 *17 allele and an LoF allele from analyses, because the metabolic phenotypes of these combinations are unknown.

Statistical methods. The computer-based analysis program SPSS (Statistical Package for the Social Sciences, 12.0 for PC, SPSS, Inc., Chicago, Illinois) was used for statistical calculations. Categorical variables are reported as counts (percentages), and continuous variables are reported as mean ± SD. We tested differences between groups with the chi-square test for categorical variables (or Fisher exact test when any expected cell count was <5 for a 2 × 2 table) and with an unpaired Student *t* test or 1-way analysis of variance for continuous variables. The Kolmogorov-Smirnov test was used to test for a normal distribution. Linear and multiple logistic regression analyses (forward conditional) with variables with a p value of <0.20 on univariate analysis were applied to estimate the association between proposed variables and the change in OTR and the persistence of high OTR with high-dose maintenance therapy. A p value <0.05 was considered significant.

The primary end point of the study was the change in OTR with clopidogrel 150 mg/day. We estimated that, assuming an SD in OTR of 80 PRU (3,20), a total of 40 subjects would provide a 90% power to see an effect size of 0.50 (i.e., a difference of 40 PRU) with a 1-sided Student *t* test. Assuming a *CYP2C19* LoF allele carrier rate of approximately 50% in patients with high OTR (15), this sample size would also provide a 70% power to see a 40-PRU difference in carriers compared with noncarriers.

Results

Baseline characteristics of the study population. A total of 41 subjects were enrolled. The baseline characteristics of the study population are listed in Table 1. In brief, 35 patients (85%) were men, the average age was 66.6 ± 10.6 years, and 16 patients (39%) had diabetes mellitus. The mean OTR on standard clopidogrel therapy was 285 ± 47 PRU.

Pharmacodynamic effect of clopidogrel 150 mg/day. The median duration of therapy with clopidogrel 150 mg/day was 8 days (range 7 to 12 days). The OTR was significantly reduced from 285 ± 47 PRU to 220 ± 91 PRU ($p < 0.001$), with a mean decrement of 65 ± 68 PRU and a mean relative reduction of 24 ± 24%.

***CYP2C19* status of the study population.** Table 2 shows the *CYP2C19* genotype distribution in the study population. The allele frequencies of *1, *2, *3, *4, and *17 were 0.60, 0.24, 0.024, 0.012, and 0.122, respectively. The variants *5, *6, *7, and *8 were not observed. A total of 20 subjects (49%) had at least 1 *CYP2C19* LoF allele. With respect to *CYP2C19* enzymatic function, 21 subjects (52.5%) were ultra-rapid or extensive metabolizers (*17/*17, *1/*17, or *1/*1), 16 subjects (40%) were intermediate metabolizers (*1/*2, *1/*3, *1/*4), and 3 subjects (7.5%) were poor metabolizers (*2/*2, *2/*3). One subject with *2/*17 genotype was not included in the analysis, because the relationship between this allelic combination and *CYP2C19* enzymatic function is unknown.

***CYP2C19* status and pharmacodynamic effect of high maintenance-dose clopidogrel.** There was no difference in baseline OTR between carriers of at least 1 *CYP2C19* LoF allele and noncarriers (292 ± 40 PRU vs. 281 ± 54 PRU, p = 0.5). The reduction in OTR with clopidogrel 150

Table 2. Frequency of *CYP2C19* Genotypes in Study Population

CYP2C19 Genotype	Frequency
*1/*1	15 (36.6%)
*1/*2	14 (14.1%)
*1/*3	1 (2.4%)
*1/*4	1 (2.4%)
*1/*17	3 (7.3%)
*17/*17	3 (7.3%)
*2/*2	2 (4.8%)
*2/*3	1 (2.4%)
*2/*17	1 (2.4%)

The genotypes *2/*4, *3/*3, *3/*4, and *4/*4 were not observed in the study population.

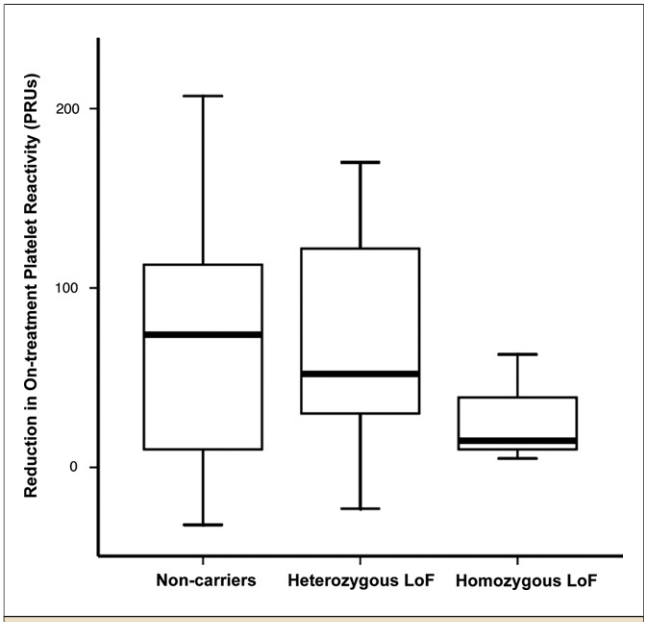

Figure 1. *CYP2C19* Status and Change in Platelet Reactivity

Reduction in on-treatment platelet reactivity with clopidogrel 150 mg/day in subjects with high on-treatment reactivity to standard-dose clopidogrel, stratified by carriage of no CYP2C19 loss-of-function (LoF) alleles or 1 or 2 CYP2C19 LoF alleles. P = 0.4 between noncarriers and carriers (heterozygous LoF and homozygous LoF).

mg/day was not significantly different between carriers of at least 1 LoF allele and noncarriers (52 ± 66 PRU vs. 77 ± 72 PRU, p = 0.25). The reduction in OTR was not significantly different according to genotype, although patients homozygous for *CYP2C19* LoF alleles had the least reduction in reactivity (*17/*17 or *1/*17 genotypes: 61 ± 75 PRU; wild-type [*1/*1]: 84 ± 72 PRU; heterozygotes [*1 with an LoF allele *2, *3, *4, *5, *6, *7, or *8]: 56 ± 71 PRU; 2 LoF alleles: 28 ± 31 PRU, p = 0.5) (Fig. 1).

Clinical characteristics and pharmacodynamic effect of high maintenance-dose clopidogrel. The relationships between the baseline clinical characteristics in Table 1 and the change in OTR were first tested by univariate analysis. Diabetic subjects tended to have a smaller reduction in OTR with high-dose maintenance clopidogrel compared with nondiabetic subjects (41 ± 76 PRU vs. 81 ± 60 PRU, p = 0.067). There was a significant negative correlation between body mass index (BMI) and the reduction in OTR (r = −0.41, p = 0.009), and there was a negative trend between creatinine clearance and the reduction in OTR (r = −0.29, p = 0.068). The concomitant use of proton-pump inhibitors (PPIs) had no effect on the response to high-dose maintenance therapy (reduction in OTR of 76 ± 73 PRU with PPIs vs. 63 ± 68 PRU without PPIs, p = 0.6). No other baseline characteristics were associated with the change in OTR after clopidogrel 150 mg/day, including the level of OTR at the start of study drug treatment.

Multivariate analysis was performed to determine the characteristics independently associated with the change in OTR. Variables that, upon univariate analysis, had a p value <0.20 were entered into the model (diabetes, creatinine clearance, and BMI). Only BMI was independently associated with the change in OTR with high-dose maintenance therapy (p = 0.009).

Effect of clopidogrel 150 mg/day on high on-treatment platelet reactivity. The OTR was "normalized" (PRU <235) by clopidogrel 150 mg/day in 23 subjects (56%). Table 3 shows the relationships among clinical characteristics as well as *CYP2C19* LoF allele carriage with persistently high OTR. Subjects with persistently high OTR were more frequently diabetic (p = 0.02), had a higher level of OTR with standard clopidogrel therapy before high-dose administration (p = 0.001), and tended to have a greater BMI (p = 0.09). The OTR on high-dose therapy was significantly correlated with OTR on standard-dose therapy (r = 0.68, p < 0.001). On multivariate analysis with the variables in Table 3 with a p value of <0.20, a higher level of OTR on standard clopidogrel was the only independent predictor of persistently high OTR with clopidogrel 150 mg/day (odds ratio: 1.029, 95% confidence interval: 1.01 to 1.05, p = 0.008). Diabetes mellitus tended to be associated with persistently high OTR on multivariate analysis, but this relationship was not statistically significant (p = 0.07).

Discussion

This is the first study to specifically examine the antiplatelet effect of increased maintenance-dose clopidogrel according to *CYP2C19* polymorphism status in a cohort of patients with high OTR with a diagnostic threshold that has been

Table 3. Relationship Between Persistently High OTR With Clopidogrel 150 mg/day and Baseline Characteristics

Characteristic	"Normalized" OTR (n = 23)	Persistently High OTR (n = 18)	p Value
Age	66.4 ± 9.6	66.8 ± 12.0	0.90
Male sex	19 (82.6%)	16 (88.9%)	0.70
Diabetes mellitus	5 (21.7%)	11 (61.1%)	0.02
Hypertension	20 (87.0%)	18 (88.9%)	1.00
Hypercholesterolemia	21 (58.3%)	15 (41.7%)	0.60
Creatinine clearance (ml/min)	75.6 ± 24.0	86.9 ± 41.1	0.28
Previous CVA	4 (17.4%)	0 (0%)	0.12
Previous MI	7 (30.4%)	6 (33.3%)	1.00
Previous CABG	7 (30.4%)	8 (44.4%)	0.50
PPI use	6 (26.1%)	4 (22.2%)	1.00
Body mass index (kg/m^2)	27.9 ± 2.8	30.9 ± 7.5	0.09
Index OTR (PRU)	265 ± 28	312 ± 54	0.001
CYP2C19 LoF allele carrier	10 (45.5%)	9 (50%)	1.00

CABG = coronary artery bypass graft surgery; CVA = cardiovascular accident; LoF = loss-of-function; MI = myocardial infarction; OTR = on-treatment platelet reactivity; PPI = proton-pump inhibitor; PRU = P2Y12 reaction units.

shown to predict adverse clinical outcomes after PCI. Our major findings are: 1) clopidogrel 150 mg/day significantly reduces platelet reactivity in the overall population of patients with high OTR on standard therapy; 2) the incremental inhibition provided by 150 mg/day is largely similar regardless of *CYP2C19* LoF polymorphism carrier status, but the change in platelet reactivity was minimal and seemed to be less in patients homozygous for *CYP2C19* LoF allele than that observed with wild-types or heterozygotes; 3) BMI is independently and negatively associated with the degree of incremental inhibition provided by the higher dose; and 4) a heightened level of reactivity on standard dosing is a predictor of persistently high OTR, despite an increased dose. These findings might have substantial clinical implications for determining the appropriate type and/or dose of $P2Y_{12}$ antagonist after PCI.

High OTR as determined by the VerifyNow P2Y12 assay has been shown to be prognostic of cardiovascular risk after PCI by several prospective studies (3–6,20), but the optimal pharmacologic strategy to increase platelet inhibition in this group of patients is not known. In this pharmacodynamic study, we observed that clopidogrel 150 mg/day significantly reduced platelet reactivity in patients with high OTR on standard therapy. Several studies have demonstrated that high-dose maintenance therapy reduces platelet reactivity compared with standard dose therapy in an unselected cohort (23–25). In small studies that used various platelet function assays and definitions of suboptimal responsiveness, an increased maintenance seemed to provide further inhibition (26–29) but not to the level of good responders (28,29). Our findings in a cohort of patients with CAD with a point-of-care platelet function assay and a well-defined optimal cut-point for high OTR are consistent with these previous studies.

Because the *CYP2C19* metabolic phenotype seems to dictate the efficiency of clopidogrel active metabolite generation (17), we examined whether *CYP2C19* LoF alleles might impact the antiplatelet effect of high-dose therapy in patients with high OTR to standard therapy. This was a comprehensive analysis and included *CYP2C19* genotyping for the *2, *3, *4, *5, *6, *7, *8, and *17 single nucleotide polymorphisms. In a nonselected population, carriers of *CYP2C19* *2 and *4 alleles have been shown to be responsive to clopidogrel 150 mg/day (25), and the inhibitory responses to sequential 600-mg loading doses were similar in *CYP2C19* *2 allele carriers and noncarriers (30). We did not observe, in our selected population of patients with high OTR, a statistically significant association between carriage of at least 1 *CYP2C19* LoF allele and the magnitude of reduction in platelet reactivity with clopidogrel 150 mg/day. However, we did see a trend that patients with 2 *CYP2C19* LoF alleles had the least reduction in reactivity. Our findings are strengthened by the inclusion of a broad spectrum of genetic variants that affect *CYP2C19* metabolism in addition to the *2 allele.

The U.S. Food and Drug Administration added a boxed warning to the clopidogrel label in March 2010 that noted that tests are available to identify the *CYP2C19* genotype of a patient. Furthermore, the warning states to "consider alternative treatment or treatment strategies in patients identified as *CYP2C19* poor metabolizers," referring to an unpublished study of healthy volunteers that showed "improved antiplatelet response" in 10 poor metabolizers treated with clopidogrel 150 mg/day (31). We observed that the antiplatelet effect of an increased clopidogrel dose seemed minimal in a small number of CAD patients with high OTR who were *CYP2C19* poor metabolizers. However, our findings are consistent with the U.S. Food and Drug Administration position on *CYP2C19* genotyping, because alternative P2Y12 inhibitors such as prasugrel and ticagrelor are not affected by *CYP2C19* status (16,17,32), and therefore systematic *CYP2C19* genotyping in patients undergoing platelet function testing could help select the appropriate P2Y12 inhibitor by identifying patients with high OTR who are poor metabolizers (i.e., patients with 2 copies of *CYP2C19* *2, *3, *4, *5, *6, *7, and *8 alleles). The impact of our findings must be tempered by the small sample size of our study. Given the low prevalence of poor metabolizers in the population, only very large trials will be able to definitively assess the pharmacodynamic effect of increased-dose clopidogrel in poor metabolizers with high OTR. The Genotype Information and Functional Testing study (NCT00992420), a substudy of the GRAVITAS (Gauging Responsiveness with A VerifyNow Assay: Impact on Thrombosis and Safety) trial (33), will help address this question.

Drug under-dosage has been proposed as an etiology of high OTR in patients with greater BMI. High BMI is a predictor of a poor response to a clopidogrel 300-mg loading dose (34,35) and was a predictor of the inability of repeated daily clopidogrel loading doses to achieve a vasodilator-stimulated phosphoprotein platelet reactivity index $<50\%$ in a small study using an unselected patient cohort (30). We observed that greater BMI was independently and negatively associated with the incremental reduction in OTR with increased maintenance-dose clopidogrel in patients with high OTR on standard treatment. Therefore, our findings question whether even an increased dosage can achieve adequate levels of platelet inhibition in obese patients with high OTR.

In clopidogrel-naïve patients, the level of post-treatment reactivity is associated with pre-treatment reactivity (36). We found that the change in reactivity with 150 mg/day was not dependent upon the level of reactivity on standard therapy. However, a greater level of reactivity on standard therapy independently predicted persistently high OTR, despite the increased dose. For every 10-PRU increase in

OTR on standard therapy, the odds for persistently high OTR increased by 29%. Therefore, if the goal of intensified antiplatelet therapy is to "normalize" platelet reactivity to below the apparent diagnostic cutoff of the VerifyNow P2Y12 device, the use of alternative P2Y12 antagonists that provide consistent and powerful inhibition of the P2Y12 receptor despite a poor clopidogrel response (37,38) might be preferred in patients with very high OTR.

Study limitations. This study has several limitations. First, it was performed as a nonrandomized, observational, single-center study. Second, the study was not adequately powered to detect differences in antiplatelet effect in poor metabolizers, and therefore our findings with regard to the minimal effect in this sub-group might be due to chance. However, we were adequately powered to see an effect size in carriers of at least 1 LoF allele (i.e., the combination of heterozygotes and homozygotes) that would likely be clinically significant. Third, although data support the relationship between high OTR and cardiovascular risk after PCI, the clinical efficacy of antiplatelet therapy adjustment on the basis of platelet function testing has not been established.

Conclusions

In patients with high OTR despite standard clopidogrel therapy, clopidogrel 150 mg/day provides a significant reduction in the magnitude of platelet reactivity. Increasing BMI is an independent predictor of poor incremental antiplatelet effect with the higher dose, and patients with higher levels of OTR to standard dosing are most likely to have persistently high OTR even with an increased dose. In the few patients that were homozygous for a *CYP2C19* LoF allele, the antiplatelet effect with the higher dose of clopidogrel seemed to be minimal. Therefore, higher-dose clopidogrel therapy improves antiplatelet response in patients with high OTR on standard therapy, but this effect might be suboptimal in obese patients and those with very high OTR.

Acknowledgments

This study was supported by the Clinical Translational Science Award National Institutes of Health/National Center for Research Resources/Scripps Translational Science Institute U54 RR025744.

Reprint requests and correspondence: Dr. Colin M. Barker, University of Texas Medical School at Houston, Internal Medicine, Division of Cardiology, 6341 Fannin Street, MSB 2.146, Houston, Texas 77030. E-mail: colin.m.barker@uth.tmc.edu.

REFERENCES

1. Barker CM, Price MJ. Antiplatelet therapy in acute coronary syndromes. Curr Cardiol Rep 2008;10:327–33.
2. Price MJ, Coleman JL, Steinhubl SR, Wong GB, Cannon CP, Teirstein PS. Onset and offset of platelet inhibition after high-dose clopidogrel loading and standard daily therapy measured by a point-of-care assay in healthy volunteers. Am J Cardiol 2006;98:681–4.
3. Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J 2008;29:992–1000.
4. Patti G, Nusca A, Mangiacapra F, Gatto L, D'Ambrosio A, Di Sciascio G. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. J Am Coll Cardiol 2008;52:1128–33.
5. Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. Circulation 2009;119:237–42.
6. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA 2010;303:754–62.
7. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol 2005;46:1820–6.
8. Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. J Am Coll Cardiol 2009;53:849–56.
9. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. Thromb Haemost 2007;97:282–7.
10. Frere C, Cuisset T, Quilici J, et al. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. Thromb Haemost 2007;98:838–43.
11. Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J 2006;27:2420–5.
12. Pereillo JM, Maftouh M, Andrieu A, et al. Structure and stereochemistry of the active metabolite of clopidogrel. Drug Metab Dispos 2002;30:1288–95.
13. Marin F, Gonzalez-Conejero R, Capranzano P, Bass TA, Roldan V, Angiolillo DJ. Pharmacogenetics in cardiovascular antithrombotic therapy. J Am Coll Cardiol 2009;54:1041–57.
14. Hulot J-S, Bura A, Villard E, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006;108:2244–7.
15. Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol 2008;51:1925–34.
16. Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 2007;5:2428–36.
17. Varenhorst C, James S, Erlinge D, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin-treated patients with coronary artery disease. Eur Heart J 2009;30:1744–52.
18. Shuldiner AR, O'Connell JR, Bliden KP, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA 2009;302:849–57.
19. Price MJ. Bedside evaluation of thienopyridine antiplatelet therapy. Circulation 2009;119:2625–32.
20. Price MJ, Nayak KR, Barker CM, Kandzari DE, Teirstein PS. Predictors of heightened platelet reactivity despite dual-antiplatelet therapy in patients undergoing percutaneous coronary intervention. Am J Cardiol 2009;103:1339–43.

21. Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med 2009;360:354–62.
22. CYP2C19 allele nomenclature. Available at: http://www.cypalleles.ki.se/cyp2c19.htm. Accessed September 30, 2009.
23. von Beckerath N, Kastrati A, Wieczorek A, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J 2007;28:1814–9.
24. Angiolillo DJ, Bernardo E, Palazuelos J, et al. Functional impact of high clopidogrel maintenance dosing in patients undergoing elective percutaneous coronary interventions. Results of a randomized study. Thromb Haemost 2008;99:161–8.
25. Gladding P, Webster M, Zeng I, et al. The pharmacogenetics and pharmacodynamics of clopidogrel response: an analysis from the PRINC (Plavix Response in Coronary Intervention) trial. J Am Coll Cardiol Intv 2008;1:620–7.
26. Aleil B, Jacquemin L, De Poli F, et al. Clopidogrel 150 mg/day to overcome low responsiveness in patients undergoing elective percutaneous coronary intervention. J Am Coll Cardiol Intv 2008:631–8.
27. Matetzky S, Fefer P, Shenkman B, Varon D, Savion N, Hod H. Effectiveness of reloading to overcome clopidogrel nonresponsiveness in patients with acute myocardial infarction. Am J Cardiol 2008;102:524–9.
28. Angiolillo DJ, Costa MA, Shoemaker SB, et al. Functional effects of high clopidogrel maintenance dosing in patients with inadequate platelet inhibition on standard dose treatment. Am J Cardiol 2008;101:440–5.
29. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation 2007;115:708–16.
30. Bonello-Palot N, Armero S, Paganelli F, et al. Relation of body mass index to high on-treatment platelet reactivity and of failed clopidogrel dose adjustment according to platelet reactivity monitoring in patients undergoing percutaneous coronary intervention. Am J Cardiol 2009;104:1511–5.
31. Plavix package insert. Available at: http://www.plavix.com. Accessed March 30, 2010.
32. van Giezen J, Nilsson L, Berntsson P, et al. Ticagrelor binds to human P2Y(12) independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. J Thromb Haemost 2009;7:1556–65.
33. Price MJ, Berger PB, Angiolillo DJ, et al. Evaluation of individualized clopidogrel therapy after drug-eluting stent implantation in patients with high residual platelet reactivity: design and rationale of the GRAVITAS trial. Am Heart J 2009;157:818–24, 824 e1.
34. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weight adjusted? J Invasive Cardiol 2004;16:169–74.
35. Ang L, Palakodeti V, Khalid A, et al. Elevated plasma fibrinogen and diabetes mellitus are associated with lower inhibition of platelet reactivity with clopidogrel. J Am Coll Cardiol 2008;52:1052–9.
36. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908–13.
37. Brandt JT, Payne CD, Wiviott SD, et al. A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. Am Heart J 2007;153:66.e9–16.
38. Gurbel PA, Bliden KP, Butler K, et al. Response to ticagrelor in clopidogrel nonresponders and responders and effect of switching therapies: the RESPOND study. Circulation 2010;121:1188–99.

Key Words: clopidogrel ■ *CYP2C19* ■ platelet ■ thienopyridine.